

Independent control of transcription initiations from two sites by an initiator-like element and TATA box in the human *c-erbB-2* promoter

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Abstract

Transcription of the human *c-erbB-2* proto-oncogene starts mainly at two sites, nucleotide positions +1 and –69. The present studies have identified an initiator-like element that specifies the position of transcription initiation at position –69. This initiator-like element contains six GGA repeats and is located just downstream from the transcription start site between positions –68 and –45. In addition, both in vitro and in vivo studies indicated that transcription initiation at position +1 is specified by a TATA box 25 bp upstream from the transcription startpoint. Thus, initiation at two sites in the *c-erbB-2* promoter is controlled independently by the initiator-like element and the TATA box.

Key words: *c-erbB-2* proto-oncogene; Transcriptional initiation; Initiator; TATA box

1. Introduction

The transcription of eukaryotic genes by RNA polymerase II is regulated through two types of DNA elements: core promoter elements at or near the transcriptional start site and regulatory elements in more distal promoter or enhancer regions (reviewed in [33]). A variety of sequence-specific activators or repressors interact with enhancers (reviewed in [28,34]), while essentially general initiation factors bind to core promoters (reviewed in [33,44]). Studies on various class II promoters indicate that they can be divided into several classes – those containing TATA elements, those containing initiator elements, those containing both elements, and the remaining promoters, which have neither TATA nor common recognizable initiator elements. In the TATA-containing promoters, transcription usually starts about 30 bp downstream of the TATA box, and deletion of the TATA box can result in spurious initiations and a low level of transcription [16,17,18,44,57]. A TATA element is recognized by TATA box-binding factor TFIID [36,43], which is a multisubunit protein complex [12,30,53,54,64], and the resulting complex provides the recognition signal for association of RNA polymerase II and the other general transcription factors [5,10,14,24,36,55]. Pyrimidine-rich initiator elements which overlaps or are close to transcription start sites were

identified in various promoters such as H2A histone [16], terminal deoxynucleotidyl transferase (TdT) [51], and adenovirus major late (AdML) [52] promoters. A sequence is not strictly conserved in many of the known initiator elements, and the distribution and diversity of initiator-like elements among various promoters is not yet clear [4,19,37,39,51,52]; (reviewed in [59]). The initiator element is sufficient to direct accurate transcription initiation in the TATA-less promoters [51]. A 120-kDa protein termed TFII-I was recently demonstrated to bind to the initiator element of the AdML promoter, to direct the general transcription machinery at its binding site [42]. In addition, the initiator of the Ad2 IVa2 promoter is recognized by the YY1 protein (also termed δ , NF-E1 or UCRBP) (for review, see [59]), which is required for efficient transcription in vitro [46,48]. In a group of promoters, both the TATA and initiator elements are located together and act in concert with each other. For example, both elements are required for efficient transcription from the *gfa* promoter, and the partially purified TFIID appears to contain a factor(s) that interacts with the initiator region in the *gfa* promoter [38]. However, the role of each element in the promoter containing both the TATA and initiator elements may vary among various promoters. In fact, TATA and the TdT initiator appeared to act independently of each other in the artificially constructed promoter [39]. To understand the role of each element, the analysis of the role of TATA and initiator elements in various promoters containing both elements are required.

The *c-erbB-2* (HER-2) proto-oncogene [9,45] is the

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human homolog of the rat *neu* gene [47] and encodes a 185-kDa glycoprotein that is related to, but distinct from, the epidermal growth factor receptor [3,62]. The *c-erbB-2* gene product has a tyrosine kinase activity [1]. Recently, a 44-kDa protein termed Neu differentiation factor (NDF) or heregulin that stimulates the kinase activity of the *neu/c-erbB-2* gene product was isolated [23,40]. However, whether NDF directly interacts with the *c-erbB-2* gene product or interacts with a closely related receptor or with a heterodimer of *c-erbB-2* protein and related molecule still remains uncertain [41]. Amplification and overexpression of the *c-erbB-2* gene were frequently detected in human adenocarcinomas, especially in breast and stomach cancers [2,29,45,55,63]. Patients having breast cancers with amplification of the *c-erbB-2* gene have a shorter time to relapse as well as a shorter overall survival [49,50]. These results suggest that a high level of *c-erbB-2* expression contributes to tumor progression. In some human mammary tumor cell lines, an enhanced *c-erbB-2* expression can occur in the absence of gene amplification, suggesting that a different molecular mechanism other than a gene amplification result in overexpression of the *c-erbB-2* mRNA in some types of tumor cells [31]. As a first step to understand the transcriptional control of the human *c-erbB-2* proto-oncogene, we had identified and characterized the *c-erbB-2* promoter region [26]. The *c-erbB-2* promoter initiates transcription mainly at two tightly clustered start sites, nucleotides +1 and -69. Since the *c-erbB-2* promoter contains a TATA element at -25, the transcription initiation at +1 appears to be directed by a TATA element. However, the mechanism of the transcription initiation at -69 is not understood.

Here, we show that the sequence, which is located downstream of the RNA start site at -69, is an initiator-like element for the transcription initiation at -69. Interestingly, the initiator-like element and the TATA element independently direct transcription initiation at -69 and +1, respectively, in the *c-erbB-2* promoter.

2. Materials and methods

2.1. Construction of plasmids

The plasmid pEBCAT4, in which the 250-bp *PstI-SamI* (nucleotides -213 to +39) containing the human *c-erbB-2* promoter is linked to the chloramphenicol acetyltransferase (CAT) gene, was described previously [26]. To construct the pEBCAT4 Δ Inr, pEBCAT4 Δ TATA, and pEBCAT4 Δ +2/+10 mutants, a deletion was introduced by the polymerase chain reaction (PCR) method [22] as follows. The *HindIII* fragment of pEBCAT4 which contains the *c-erbB-2* promoter was cloned into pUC12. The generated plasmid pUCEB4 was used as a target DNA for PCR. Six oligonucleotides that contain sequences on either side of the region to be deleted were synthesized. They are 5'-CTTGCTCCCAATCACA---GGGCTGCTTGAGGAAG-3' (Δ Inr), the sequence complementary to Δ Inr (Δ Inr-R), 5'-GGGCTGCTTGAGGAAG---GAATGAAGTTGTGAAG-3' (Δ TATA), the sequence complementary to Δ TATA (Δ TATA-R), 5'-TTGTGAAGCTGACATT---TGGGACCGGAGAAACC-3' (Δ +2/+10), and the sequence complementary to Δ +2/+10 (Δ +2/+10-R). Two oligonucleotides

(M4 and RV) that contain sequences of the region outside of the cloning sites of pUC12 were obtained from Takara Shuzo Co. Ltd. To introduce the deletion of the initiator-like sequence around -69 (Δ Inr), two PCRs were first done separately with primers Δ Inr and RV or Δ Inr-R and M4. The products are separated from excess primers, and mixed, denatured, and allowed to reanneal. Some of the molecules recombine through the overlap made by the primers Δ Inr and Δ Inr-R. By using these reannealed DNAs as a target, PCR was done with the outside primers M4 and RV. The amplified DNA was digested with *HindIII* and cloned into pSV0CAT. The generated clone was confirmed to contain the deletion of the initiator-like sequence around -69 by DNA sequencing. The deletion of the TATA element (Δ TATA) or the initiator-like sequence around +1 (Δ +2/+10) was also introduced in a similar way.

2.2. DNA transfection and RNA analysis

Mixtures of 6 μ g of each CAT plasmid DNA, 6 μ g of pUC19 DNA, and 2 μ g of pRSV- β -gal plasmid DNA [13] were transfected into CV-1 cells by the CaPO₄ method as described [15]. The plasmid pRSV- β -gal, which carries the *E. coli* β -galactosidase gene linked to the LTR promoter of Rous sarcoma virus, was used as an internal control for differences in transfection efficiency between precipitates. To analyze CAT RNA, RNA was isolated by the guanidium thiocyanate method [7] 40 h after transfection. At the same time β -galactosidase activity was measured using a sample of cells to confirm that transfection efficiencies were similar. Primer extension analysis was done as described by Muhich et al. [35] with 50 μ g of poly(A)⁺ RNA. A synthetic 24-base oligonucleotide (5'-GCCATTGGGATATATCAACGGTGG-3') complementary to nucleotides 26–49 of the coding sequence of the CAT gene was used as a primer. The amount of the extended primer was measured by a Bioimage Analyzer (Fuji Photo Film Co. Ltd.).

2.3. DNase I footprint analyses with human TFIID

The *HindIII-HaeII* fragment (nucleotides +39 to -192) of pEBCAT1 [26], in which the 530-bp DNA fragment containing the *c-erbB-2* promoter was linked to the CAT gene, was ³²P-labeled at the 5'-end of the *HindIII* site with T4 polynucleotide kinase. The binding reaction was done by using approximately 5 ng of ³²P-labeled DNA fragment in 25 μ l reaction mixture containing 12 mM Tris-HCl (pH 7.7 at 4°C), 40 mM HEPES (pH 8.4), 60 mM KCl, 12% (v/v) glycerol, 4 mM MgCl₂, 2 μ g/ml poly(dG-dC), 0.1 mg/ml bovine serum albumin, and 0.6 μ g of human TFIID (ω -amino octyl agarose fraction) [35,36]. The DNase I digestion was done with 5 μ g/ml DNase I for 30 s at 30°C, and then stopped by the addition of 75 μ l of a solution containing 12 mM EDTA and 40 μ g/ml of tRNA. The DNA was extracted by phenol/chloroform followed by ethanol precipitation, and then analyzed on an 8% sequencing gel.

2.4. In vitro transcription

HeLa cells nuclear extract was prepared essentially as described by Dignam et al. [11]. Heat-treated nuclear extract was prepared as described by Nakajima et al. [36]. Fifteen μ l of HeLa nuclear extract was incubated with 0.5 μ g of supercoiled template DNA in 25 μ l of reaction containing 12 mM Tris-HCl (pH 7.9), 20 mM HEPES (pH 8.4), 12% (v/v) glycerol, 60 mM KCl, 8 mM MgCl₂, and 0.5 mM each of all four ribonucleoside triphosphate. Following the incubation at 30°C for 60 min, transcription was stopped by adding 75 μ l of solution containing 450 mM sodium acetate (pH 5.2), 10 mM EDTA, 0.5% SDS, and 1 mg/ml yeast tRNA to the reaction mixture. RNA was isolated by phenol/chloroform extraction followed by ethanol precipitation. Primer extension analysis was done as described above.

3. Results

3.1. The *c-erbB-2* promoter has initiator-like sequences and a functional TATA element

To begin the analysis of the mechanism by which transcription starts at two clustered sites (+1 and -69) in the *c-erbB-2* promoter, we compared the resident nucleotide sequence (Fig. 1a) with those of other promoters con-

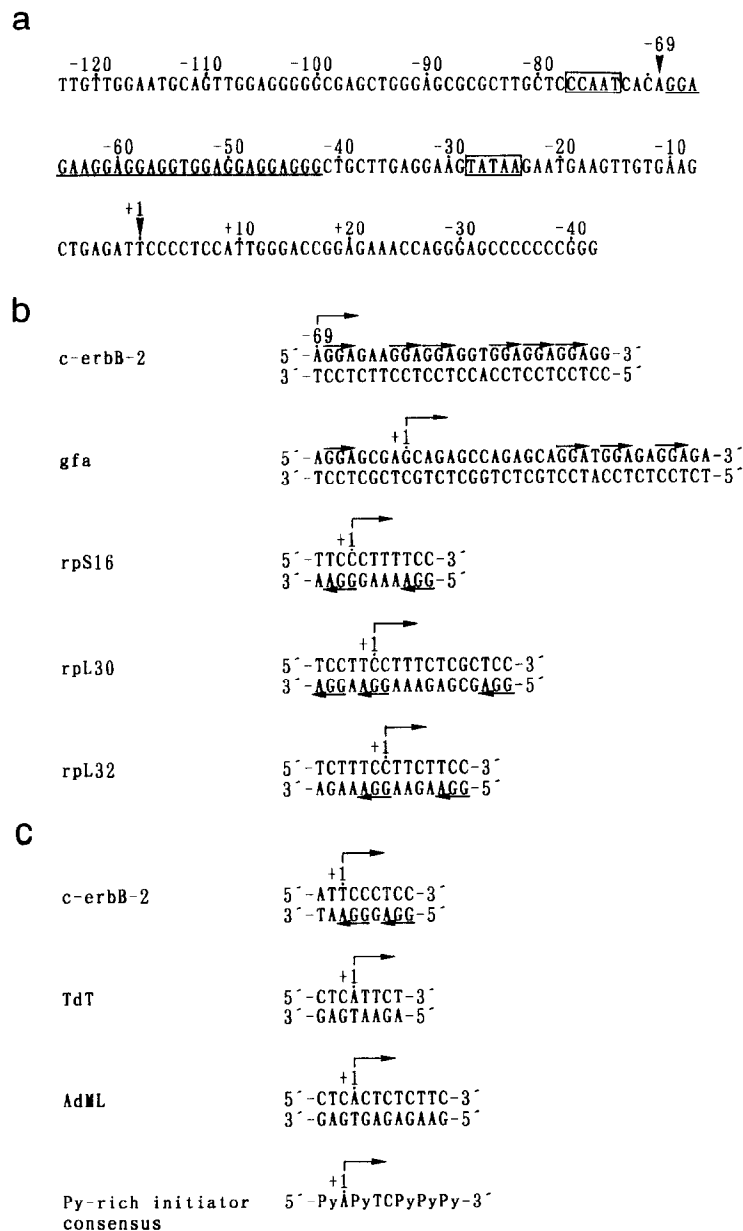


Fig. 1. The initiator-like sequences in the human *c-erbB-2* promoter. (a) Nucleotide sequence of the human *c-erbB-2* promoter region [26]. Nucleotide +1 corresponds to the farthest downstream RNA start site and residues preceding this position are represented by negative numbers. The two clustered RNA start sites are indicated by arrows. The TATA and CCAAT sequences are boxed, and the initiator-like sequences are underlined. (b) Similarity of the *c-erbB-2* initiator-like sequence around -69 and the initiator sequences of other promoters. Arrows indicate the major transcription start sites. The GGA sequence is also shown by arrows. (c) Similarity of the *c-erbB-2* initiator-like sequence around +1 and the pyrimidine-rich initiator sequences.

taining the initiator elements. A strict sequence conservation was not observed between the sequence around -69 of the *c-erbB-2* promoter and those of several known initiator elements. However, the sequence downstream of -69 in the *c-erbB-2* promoter is purine-rich and is similar to the *gfa* downstream initiator-like element (Fig. 1b). This purine-rich sequence of the *c-erbB-2* promoter consists of six GGA repeats, one GAA sequence, and one GGT sequence. The *gfa* initiator-like element that is downstream of the RNA start site also has a purine-rich

sequence, and contains three GGA repeats [37]. Some of the known initiator elements, including that of the ribosomal protein gene, have a pyrimidine-rich sequence [19], and their complementary sequences are purine-rich (Fig. 1b). Therefore, if the orientations and the positions of the initiator elements are not necessarily fixed, the purine-rich sequence in the *c-erbB-2* promoter are a good candidate for an initiator. The *c-erbB-2* promoter also has an initiator-like sequence around the downstream transcription start site at nucleotide position +1 (Fig. 1c). The

sequence around +1 in the *c-erbB-2* promoter is ATTCCCT which matches the consensus sequence for the pyrimidine-rich initiator or initiator-like element identified in promoters such as TdT and AdML promoters [39], although the initiating base of the *c-erbB-2* promoter (the second T residue) is different from those of other promoters (the A residue) in this consensus sequence. Thus, two initiator-like sequences are located in the *c-erbB-2* promoter, similar to situation described for several other promoters with single initiation site [39].

In addition to the initiator-like sequences, the *c-erbB-2* promoter also contains a TATA box which sequence is TATAA at –25. To examine whether TATA box-binding factor TFIID can bind to this TATAA element, DNase I footprint analysis was done. The TFIID purified from HeLa cells protected a region corresponding to nucleotides –18 to –31 which contains the TATAA sequence at the center (Fig. 2). Thus, the *c-erbB-2* promoter contains both the initiator-like sequences and a possibly functional TATA element.

3.2. Role of the initiator-like sequences and the TATA element in *in vitro* transcription

To study the functional role of the initiator-like sequences around –69 and +1 and the TATA element at –25 in the *c-erbB-2* promoter, and functional relationship between them, three promoter mutants lacking either of two initiator-like sequences (Δ Inr or Δ +2/+10) or the TATA element (Δ TATA) were constructed (Fig. 3). To examine the ability of the transcription initiation from the *c-erbB-2* mutant promoters, we employed an *in vitro* transcription system derived from HeLa nuclear extract with the supercoiled CAT plasmid DNA containing the wild-type promoter or a promoter having one of its elements deleted as templates (Fig. 4). The transcripts were detected by primer extension analysis with a primer of the synthetic oligonucleotide complementary to the coding sequence of the CAT gene (Fig. 4a and b). The 193- and 124-base extended primers correspond to the predicted length of *c-erbB-2*-CAT mRNA initiated from –69 and +1 in the *c-erbB-2* promoter, respectively (108 or 39 nucleotides of *c-erbB-2* mRNA plus 85 nucleotides of CAT RNA). The amount of each extended primer was measured and represented by a bar graph in Fig. 4c. A template containing the wild-type promoter generated transcripts corresponding to initiations at both –69 and +1, respectively, and the ratio of transcripts was about 1:1.4. In the following part of this paper, the amount of transcripts is shown relative to that started from –69 in pEBCAT4. From the *hsp70*-CAT control template, in which the 5' regulatory region of the human *hsp70* gene was linked to the CAT gene [61], the 235-base extended primer was generated (150 nucleotides of *hsp70* mRNA plus 85 nucleotides of CAT RNA). Deletion of the initiator-like sequence around –69 abolished the transcription initiation at –69, but not at +1. In the case of

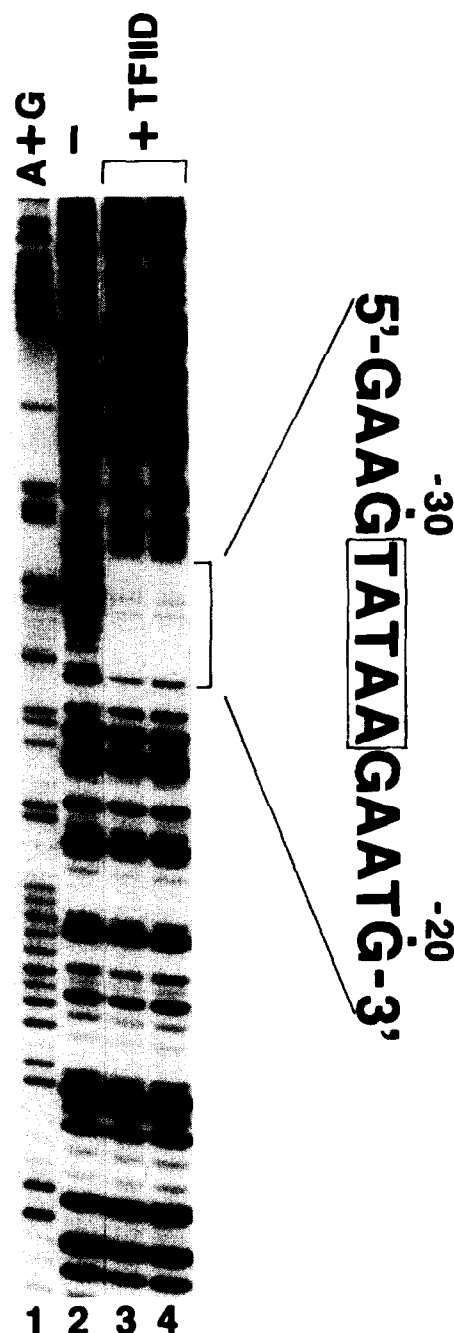


Fig. 2. DNase I footprinting analysis of TFIID binding to the TATA element of the *c-erbB-2* promoter. The *Hind*III-*Hae*II fragment of the plasmid pEBCAT1 that spanned the *c-erbB-2* promoter was 32 P-labeled at the 5'-end of the *Hind*III site. The labeled DNA fragment was incubated with 0.6 μ g of purified human TFIID (lanes 3 and 4) or no protein (lane 2). DNase I digestions were done with 3 (lanes 2 and 3) or 5 (lane 4) μ g/ml DNase I. The position of the protected region is shown on the right. A + G (lane 1) refers to the adenine and guanine marker obtained by the chemical cleavage of the same end-labeled DNA fragment.

Δ TATA, on the other hand, the transcription initiation at +1 was not observed, but the amount of transcripts started from –69 was similar to that in the wild-type

promoter. Deletion of the initiator-like sequence around +1 affected neither the transcription initiation at –69 nor +1. Thus, the initiator-like sequence containing the GGA repeats region around –69 functions as an initiator-like element for the transcription initiation at –69, whereas the initiator-like sequence around +1 does not. Furthermore, a TATA box at –25 controls the transcription initiation at +1. These results indicate that the transcription initiations at –69 and +1 are governed independently by the initiator-like element and the TATA element, respectively.

3.3. TFIID is required for the transcription initiation at +1

Mild heat treatment of a nuclear extract preferentially inactivates TFIID, and supplementation of the heat-treated extract with TFIID-containing fractions can restore AdML promoter-dependent transcription [36]. Using the human *hsp70* promoter [61] as a control (the initiation of transcription driven by this promoter is dependent on its TATA element), we asked whether the transcription initiations at –69 and +1 were TFIID dependent. Nuclear extracts were heated for 15 min at 47°C and immediately used for in vitro transcription. As shown in Fig. 5, the transcription initiation at +1 was completely abolished after this treatment whereas the transcription initiation at –69 was unaffected. Then, the purified human TFIID was added to the heat-treated extract and used for the in vitro transcription. Addition of TFIID completely restored the transcription initiation at +1. These results indicate that TFIID is required for

the transcription initiation at +1 whereas transcription initiation at –69 utilizes a heat resistant form of TFIID.

3.4. Role of the initiator-like element and TATA element in the *c-erbB-2* promoter in vivo

To confirm the in vivo roles of the initiator-like element around –69 and the TATA element at –25, CV-1 cells were transfected with the CAT plasmid containing the wild-type or mutant *c-erbB-2* promoter, and RNAs were analyzed by primer extension analysis (Fig. 6). With the wild-type promoter, the level of transcripts started from +1 was about 70% of that from –69. Deletion of the initiator-like element (Δ Inr) markedly decreased the amount of –69 transcripts, but did not affect the amount of +1 transcripts. In contrast, deletion of the TATA element (Δ TATA) almost completely abolished the transcription initiation at +1, but had no effect on the transcription initiation at –69. These in vivo results are consistent with those obtained by the in vitro transcription studies. Thus, in both in vivo and in vitro transcription assays, the initiator-like element and the TATA element independently control the transcription initiations at –69 and +1, respectively.

4. Discussion

In spite of the presence of a typical TATA box, the human *c-erbB-2* promoter has two RNA start sites, at positions +1 and –69 [26]. Since the TATA element is 25 bp upstream of the RNA start site +1, the transcription

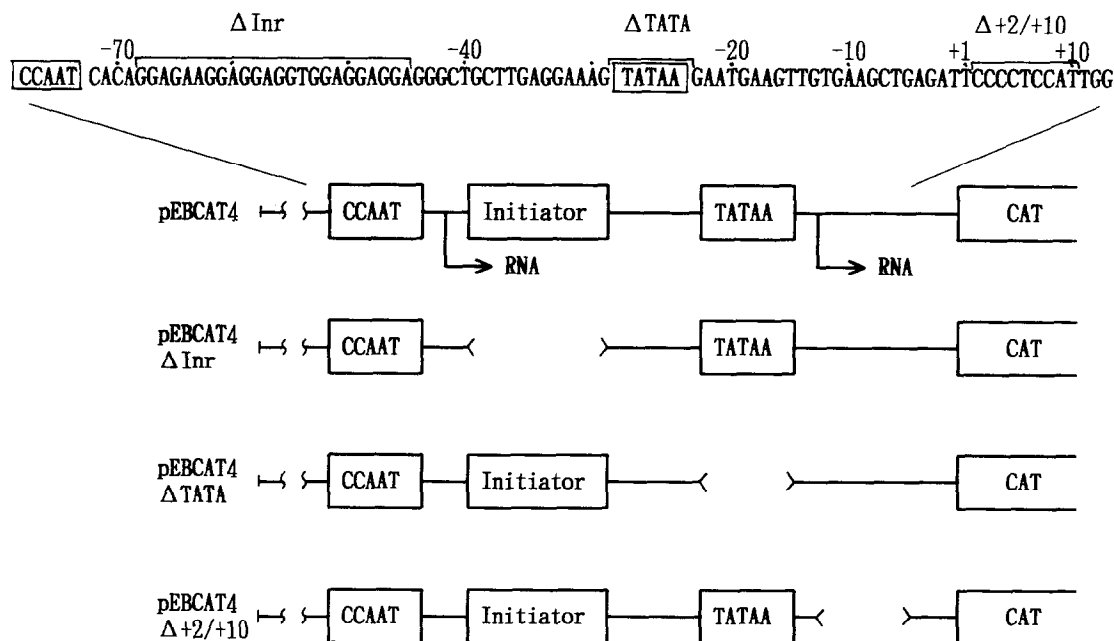


Fig. 3. Structure of the four *c-erbB-2* promoter-CAT constructs used. The plasmid pEBCAT4 contains the wild-type *c-erbB-2* promoter. The three constructs pEBCAT4 Δ Inr, pEBCAT4 Δ TATA and pEBCAT4 Δ +2/+10 are deletion mutants of the initiator-like sequence around –69, the TATA element, and the initiator-like sequence around +1, respectively. The nucleotide sequences deleted in three plasmids are indicated at the top.

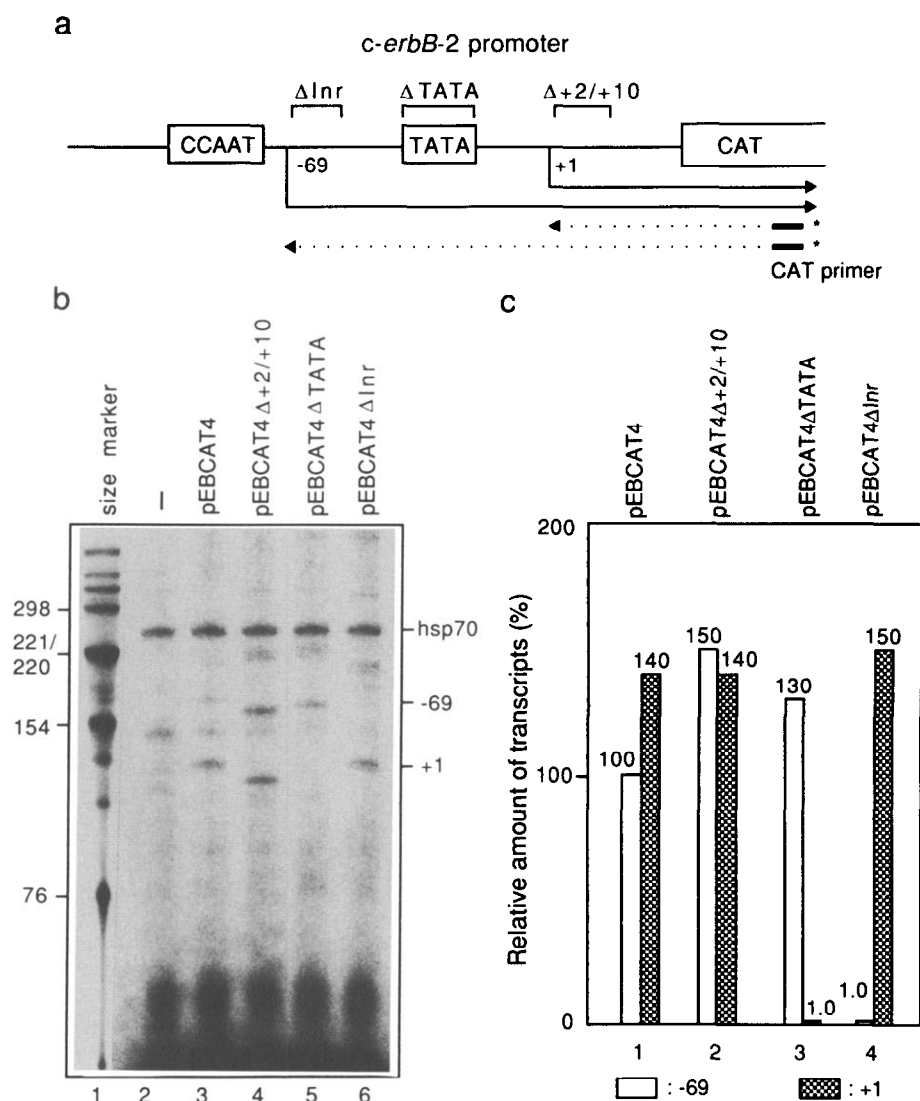


Fig. 4. In vitro transcription of the *c-erbB-2* promoter mutants. (a) A schematic representation of the primer extension analysis of CAT RNA. Primer extension analysis was done with a primer complementary to a segment of CAT mRNA. (b) In vitro transcription with various promoters. The closed circular DNA of the human *hsp70* promoter-CAT plasmid (lane 1) or a mixture of the closed circular DNAs of the *c-erbB-2* promoter-CAT construct shown above each lane and the *hsp70* promoter-CAT plasmid (lanes 3–6) was transcribed in HeLa nuclear extracts. The human *hsp70* promoter-CAT plasmid was used as the control template. The transcribed CAT RNAs were analyzed by primer extension analysis. The extended primers corresponding to the *c-erbB-2*-CAT mRNA started from -69 to +1, and that corresponding to the *hsp70*-CAT mRNA are shown by arrows. 32 P-Labeled *Hinf*I-digested pBR322 was used as a size marker (lane 1). (c) Quantitation of in vitro transcription with various promoters. The amount of extended primer generated from the *c-erbB-2*-CAT mRNA was measured, and is expressed relative to that corresponding to the *c-erbB-2* CAT mRNA started from -69 in pEBCAT4. The open and shaded bars correspond to the transcripts started from -69 and +1, respectively. The amount of each extended primer corresponding to the *c-erbB-2*-CAT mRNA was normalized with respect to that generated from the *hsp70*-CAT mRNA.

initiation from this site was assumed to be directed by the TATA element. In contrast, no mechanism of the transcription initiation from -69 has been advanced. We have identified the initiator-like element for the transcription initiation from -69, which has the features of the presence of GGA repeats and the location in downstream from the RNA start site. The most interesting property of the transcription from this *c-erbB-2* promoter is the completely independent control of transcription initiations from those two sites by the initiator-like

and TATA elements. Initiation from a single site in promoters such as the AdML promoter and the *gfa* promoter appear to require or to be influenced by both the TATA and initiator elements [20,37]. Furthermore, a combination of the initiator of the TdT gene with the TATA box of the AdML promoter produces a greatly increased level of transcription initiation at the initiator [51]. On the other hand, recently, a TATA element and the TdT initiator in the artificially constructed promoter were shown to act independently of each other [39].

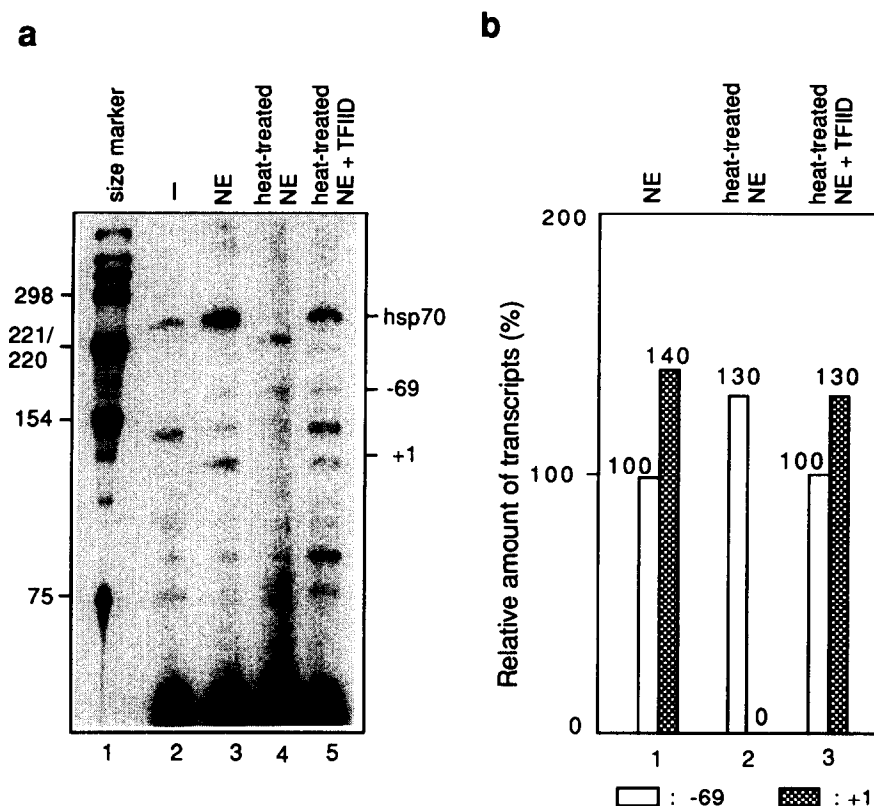


Fig. 5. Requirement of TFIID for in vitro transcription initiation at +1 in the *c-erbB-2* promoter. (a) Recovery of transcription activity of heat-treated nuclear extract by adding TFIID. The closed circular DNA of *hsp70* promoter-CAT construct (lane 2) or a mixture of the closed circular DNAs of pEBCAT4 and *hsp70* promoter-CAT construct (lanes 3–5) was used as a DNA template for in vitro transcription. The human *hsp70* promoter-CAT plasmid was used as the control template. In vitro transcription was done with HeLa nuclear extract (lanes 2 and 3), heat-treated HeLa nuclear extract (lane 4), or heat-treated HeLa nuclear extract complemented with the purified human TFIID (lane 5). Heat treatment of HeLa nuclear extract selectively inactivated the TFIID activity. The transcribed CAT RNAs were analyzed by primer extension analysis as shown in Fig. 4a. The extended primers corresponding to the *c-erbB-2*-CAT mRNA started from -69 and +1, and that corresponding to the *hsp70*-CAT mRNA are indicated by arrows. 32 P-Labeled *Hinf*I-digested pBR322 was used as a size marker (lane 1). (b) Quantitation of in vitro transcription.

However, to our knowledge, our studies about the *c-erbB-2* promoter provides the first evidence for the independent control of transcription initiation by two elements, a TATA box and an initiator-like element, in a natural promoter containing both elements.

The sequence of the *c-erbB-2* initiator-like element identified here is purine-rich and contains several GGA repeats. A similar purine-rich sequence was found in the sense strand of the *gfa* downstream initiator-like element [37] and in the opposite strand of the initiators or initiator-like elements found in promoters such as the TdT, AdML [51], and ribosomal protein gene promoters [19], but the presence of several GGA repeats is unique to the *c-erbB-2* initiator-like element. Evidence reported so far for the initiator-like elements in the ribosomal protein gene (rpS16) promoters also confirm the difference between these initiator-like elements and the *c-erbB-2* initiator-like element. Mutagenesis analysis of the rpS16 gene initiator-like element indicated that an uninterrupted stretch of pyrimidines in the region containing this element is not necessary for efficient transcription

[19]. Interestingly, several repeats of TCC, an inverted sequence of GGA, were found in several promoter regions including the EGF receptor gene [25] and the mouse *c-myc* gene [58], both of which have multiple RNA start sites. The region containing TCC repeats is known to bind nuclear proteins [27] and to be associated with S1 nuclease sensitivity [32]. If the binding of proteins to these sequences is independent of the DNA sequence orientation, the TCC repeats also may function like the *c-erbB-2* initiator-like element.

The *gfa* initiator-like element was protected from DNase digestion by HeLa TFIID, suggesting that partially purified human TFIID contains a component(s) that interacts with the *gfa* initiator-like element [38]. In contrast, HeLa TFIID protected from DNase digestion only the TATA element not the initiator-like element in the *c-erbB-2* promoter, in spite of the short distance between the two elements. Consistent with this, we recently detected a protein in HeLa nuclear extract that binds to the *c-erbB-2* initiator-like element by using gel retardation analysis, and this protein was separated from the

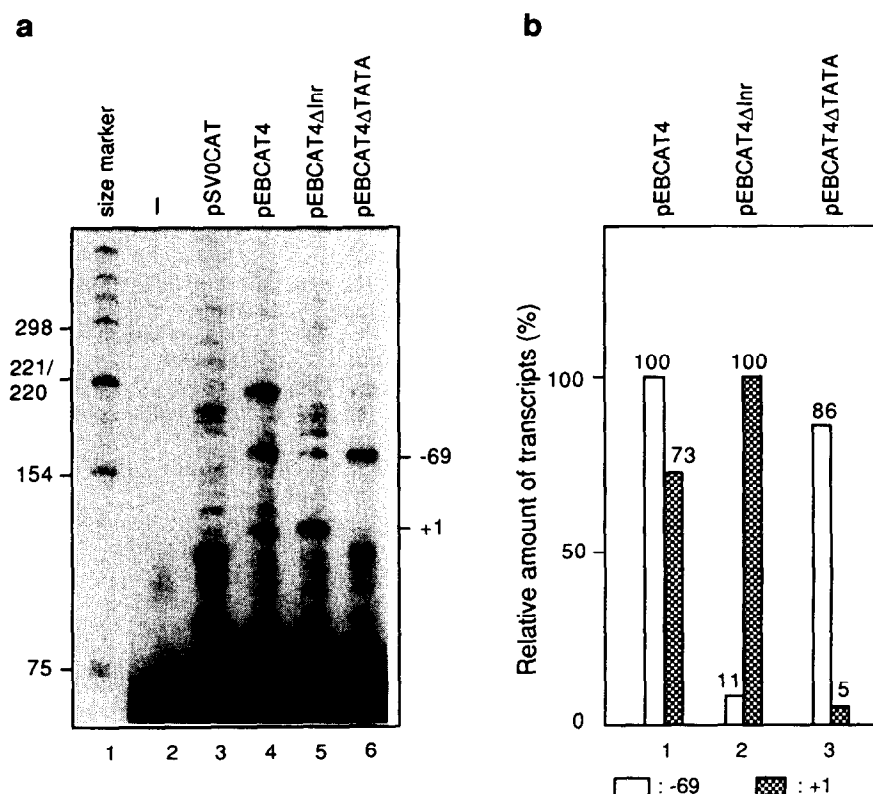


Fig. 6. Role of the initiator-like and TATA elements on the *c-erbB-2* promoter activity in vivo. (a) Primer extension analysis of CAT RNA. RNA was prepared from CV-1 cells transfected by a mixture of CAT plasmid DNA indicated above each lane and pRSV- β -gal plasmid DNA. The CAT RNAs were analyzed by primer extension analysis as shown in Fig. 4a. The extended primers corresponding to the *c-erbB-2*-CAT mRNAs started from -69 and +1 in the *c-erbB-2* promoter are indicated by arrows. 32 P-Labeled *Hinf*I-digested pBR322 was used as a size marker (lane 1). (b) Quantitation of primer extension analysis.

TFIID activity by chromatography on a phosphocellulose column (data not shown). These results strongly suggest that the protein which binds to the *c-erbB-2* initiator-like element is not associated with TFIID, unlike the protein which binds to the *gfa* initiator-like element.

Heat-inactivation of the TFIID activity suppressed the transcription initiation from position +1, but not position -69 in the *c-erbB-2* promoter. Transcription initiation in human porphobilinogen deaminase (PBGD) gene promoter, which lacks a TATA element but contains an initiator element around the initiation site, was also reported to be unaffected by heat-inactivation of the TFIID activity [4]. The TATA box-binding subunit (TBP or TFIID τ) involved in a TFIID complex plays a central role in transcription by RNA polymerase II. Following two observations suggest that TBP is positioned in initiation complexes formed on both TATA-containing and TATA-less promoters; TBP can bind with weak affinity to the -30 region of several TATA-less promoters including the TdT promoter [60], and in addition insertion of a TATA box upstream of an initiator augments transcription efficiency [6,51]. The recent finding that TBP is an integral component of the RNA polymerase I transcription factor, SL1 [8] also may support the possibility that TBP is a universal eukaryotic transcrip-

tion factor (for review, see [21]). Therefore, it is likely that a TBP-containing TFIID complex, which participates in the transcription initiation mediated by a *c-erbB-2* initiator-like element, involves some additional factor(s) which confers heat resistance.

By identification of a resident initiator-like element, the structure of the *c-erbB-2* core promoter has been clarified. Since transcription initiation events from two sites are independently controlled by TATA element and initiator-like elements, it will be interesting to determine whether the ratio of transcripts initiated from +1 and -69 is constant or variable in various cells. Furthermore, it will be important to address the contributions of the initiator and TATA elements towards mediating transcriptional activation by the activators such as the CAAT box-binding proteins that binds to the upstream region in the *c-erbB-2* promoter. Further study of the *c-erbB-2* initiator-like element and the protein(s) which binds to this element will help to understand not only the diversity of initiator but also the mechanism by which the expression of the *c-erbB-2* proto-oncogene is regulated.

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